Pak. J. Bot., 36(1): 173-180, 2004.

ISOLATION AND IDENTIFICAITON OF YEAST FLORA FROM SOIL OF KARACHI, PAKISTAN

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Abstract

On the basis of morphological and physiological/biochemical characteristics, 4 genera and 5 species of yeasts were isolated and identified from cultivated soil and 16 species belonging to 12 genera from garden soil. The identified yeast species included anamorphic and teleomorphic Ascomycetes and Basidiomycetes which have not hitherto been reported from Pakistan.

Introduction

Yeast is a group of fungi in which unicellular form is predominant. Most of the yeasts are represented in Sub Division Ascomycotina and Basidiomycotina of the kingdom Mycota. As a group of microorganisms yeasts have cosmopolitan distribution. They have been isolated from natural substrates like leaves, flowers, sweet fruits, grains, fleshy fungi, exudates of trees, insect, dung and soil (Spencer & Spencer, 1997). They play their role in the dynamics of biological and chemical turnover in soil, plants, animals and water (Rose & Harrison, 1987-1993). There are about 100 genera and 700 species of yeasts (Kurtzman & Fell, 1999) of which only 5 genera and 7 species have been reported from Pakistan (Mirza & Qureshi, 1978). It seems that no progress has been made since then.

In the present work effort has been made to study the yeast mycoflora present in two different kinds of soil. These have been identified up to species level on the basis of their morphological and physiological/biochemical characteristics.

Materials and Methods

Thirty-five soil samples were collected from a cultivated wheat field and 15 from a garden at the Karachi University campus. Yeasts associated with soil were isolated by serial dilution method (Waksman & Fred, 1922). Soil dilution made up to 10,000 were inoculated either on malt yeast glucose peptone (YM), malt extract or yeast morphology agar medium and incubated for 5-7 days at 25±1°C. Three isolates from morphologically similar looking growing colonies of yeast per plate were selected as representatives of the yeast flora. Yeast cultures were purified and maintained on yeast-morphology agar buffered at pH 4.5. All yeast isolates were primarily classified into 7 different groups viz., I-pink, II-methanol assimilating, III- cap-, hat-, saturn- or walnut- shaped ascospore producing, IV- round-, oval-, conical- or reniform shaped ascospore producing, V-ballistoconidia forming, VI-basidiomycetous and VII-glucose fermenting. Identification of yeasts up to species level was carried on the basis of standard morphological and physiological/ biochemical tests proposed for each group by Barnett *et al*., (1990) and Kurtzman & Fell (1999).

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Table 1. Yeast flora isolated from soil*.

No.	Yeast species	Cultivated soil		Garden soil	
		Occurrence %	M. cfu ± SE	Occurrence %	M. cfu±SE
1.	*Bensingtonia phyllada*	----	--- ---	2.86	0.27±0.27[a]
2.	*Bullera pyricola*	6.66	0.02±0.02[a]	----	--- ---
3.	*B. pseudoalba*	6.66	0.04±0.04[a]	----	--- ---
4.	*Candida succiphila*	----	--- ---	2.86	0.18±0.18[b]
5.	*C. valdiviana*	----	--- ---	2.86	0.02±0.02[c]
6.	*Cryptococcus albidus*	----	--- ---	5.71	0.25±0.22[a]
7.	*Debaryomyces castellii*	----	--- ---	2.86	0.01±0.01[c]
8.	*D. hansenii*	6.66	0.41±0.41[b]	5.71	0.05±0.03[c]
9.	*Debaryomyces yamadae*	----	--- ---	2.86	0.02±0.02[c]
10.	*Fibulobasidium inconspicuum*	----	--- ---	2.86	0.002±0.002[c]
11.	*Filobasidiella neoformans*	----	--- ---	2.86	0.023±0.01[c]
12.	*Filobasidium uniguttulatum*	----	--- ---	2.86	0.02±0.02[c]
13.	*Phaffia rhodozyma*	----	--- ---	2.86	0.02±0.02[c]
14.	*Pichia euphorbiae*	----	--- ---	2.86	0.02±0.02[c]
15.	*P. jadinii*	----	--- ---	2.86	0.02±0.02[c]
16.	*P. lynferdii*	6.66	0.35±0.35[c]	----	--- ---
17.	*Rhodosporidium toruloides*	----	--- ---	2.86	0.02±0.02[c]
18.	*Rhodotorula pilati*	6.66	0.26±0.26[d]	----	--- ---
19.	*Sporidiobolus ruineniae*	----	--- ---	5.71	0.25±0.24[a]
20.	*Zygosaccharomyces bailii*	----	--- ---	2.86	0.09±0.09[b]

*= Values of mean colony forming unit/g (M. cfu) and standard error (SE) are in 10,000.
Mean values in each column having different letters are significantly different at p<0.001 (Bonferoni test).

Results and Discussion

Four genera and 5 species of yeast from 35 samples of cultivated soil and 16 species of yeast belonging to 12 genera from 15 samples of garden soil (Table 1) were isolated, and identified on the basis of their morphological (Table 2) and physiological/biochemical characteristics (Table 3 & 4). *Debaryomyces castellii* Capriotti, *D. hansenii* (Zopf) Lodder & Kreger-van Rij, *D. yamadae* (van der Walt & E. Johannsen) van der Walt, M.Th. Smith & Y. Yamada, *Pichia euphorbiae* van der Walt & Opperman, *P. jadinii* (A. & R. Sartory, Weill & Mayer) Kurtzman, *P. lynferdii* (van der Walt & E. Johannsen) Kurtzman, and *Zygosaccharomyces bailii* (Lindner) Guilliermond were identified as teleomorphic ascomycetous yeast species, whereas, *Candida succiphila* J.-D. Lee & Komagata and *C. valdiviana* Grinbergs & Yarrow as anamorphic ascomycetous yeast species. On the other hand teleomorphic basidiomycetous yeast species included *Fibulobasidium inconspicuum* Bandoni, *Filobasidiella neoformans* Kwon-Chung, *Filobasidium uniguttulatum* Kwon-Chung, *Rhodosporidium toruloides* Banno, *Sporidiobolus ruineniae* Holzschu, Tredick & Phaff and anamorphic basidiomycetous yeast species were *Bensingtonia phyllada* (Nakase & M. Suzuki) Nakase & Boekhout, *Bullera pyricola* Stadelmann, *B. pseudoalba* Nakase & M. Suzuki, *Cryptococcus albidus* (Saito) C.E. Skinner, *Phaffia rhodozyma* M.W. Miller, Yoneyama & Soneda and *Rhodotorula pilati* (F.H. Jacob, Faure-Raynaud & Beron) J.A. Barnett, Payne & Yarrow. All yeast species appeared newly reported from Pakistan.

Table 2. Morphological characteristics of yeast species.

No.	Yeast species*	Group	Colony color	Shape of cell	Pseudomycelium	Septate hyphae	Ballistoconidia	Symmetric conidia	Ascospores round, oval, conical or reniform	Ascospres cap-, hat-, Saturnm- or walnut shaped
1.	*Bensingtonia phyllada*[1]	V	wh.cr.	ov-eli	-	+	+	-	-	-
2.	*Bullera pyricola*[1]	V	wh.cr.br.yl.	ov-cy	+	-	+	+	-	-
3.	*B. pseudoalba*[1]	V	wh.cr.	eli	+	+	+	+	-	-
4.	*Candida succiphila*[1]	II	wh.cr.	sgl-gl.	-	-	+	+	-	-
5.	*C. valdiviana*[1]	VII	wh.cr.	gl-ov	+	-	+	+	-	-
6.	*Cryptococcus albidus*[3]	VI	cr.	gl-ov	-	-	-	-	-	-
7.	*Debaryomyces castellii*[1]	IV	or.-pi.	r-ov	-	-	-	-	+	-
8.	*D. hansenii*[3]	IV	wh.cr.	r-ov	-	-	-	-	+	-
9.	*D. yamadae*[1]	IV	wh.cr.	r-ov	-	-	-	-	+	-
10.	*Fibulobasidium inconspicuum*[1]	VI	wh.cr.	sgl-gl.	-	-	-	-	-	-
11.	*Filobasidiella neoformans*[1]	VI	wh.cr.	sgl-gl.	-	+	-	-	-	-
12.	*Filobasidium uniguttulatum*[1]	VI	wh.-tan.	sgl-gl.	-	+	-	-	-	-
13.	*Phaffia rhodozyma*[1]	VI,VII	pi.-red	ov-elo	-	-	-	-	-	-
14.	*Pichia euphorbiae*[1]	III	wh.cr.	r-ov	-	-	-	-	-	+
15.	*P. jadinii*[1]	III	wh.cr.	sph-ov	-	-	-	-	-	+
16.	*P. lynferdii*[1]	VII	wh.cr.	sph-ov	-	-	-	-	-	+
17.	*Rhodosporidium toruloides*[1]	I	pi.-red	sph-elo	+	-	-	-	-	-
18.	*Rhodotorula pilati*[1]	VI	wh.cr.	ov	-	-	-	-	-	-
19.	*Sporidiobolus ruineniae*[2]	I	pi.	ov-cy	-	+	-	-	-	-
20.	*Zygosaccharomyces bailii*[1]	IV	t.wh.	sph-ov	+	-	-	-	+	-

Colony color: wh=white; cr=cream, yl=yellow; br=brown; tan= tan; or=orange; pi=pink; t.wh=tanish white.
Shape of cell: r=round; ov=oval; gl=globose; sgl= sub globose; sph=spherical; elo=elongated; eli=eliptical; cyl=cylindrical
*Superscript values on each species represent the number of isolates tested.

Table 3. Assimilation of carbon compounds by yeast species.

		Yeast species																		
		1	3	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
No.	**Carbon Assimilation tests**	***Bensingtonia phyllada***	***Bullera pseudoalba***	***B. pyricola***	***Candida succiphila***	***C. valdiviana***	***Cryptococcus albidus***	***Debaryomyces castellii***	***D. hansenii***	***D. yamadae***	***Fibulobasidium inconspicuum***	***Filobasidiella neoformans***	***Filobasidium uniguttulatum***	***Phaffia rhodozyma***	***Pichia euphorbiae***	***P. jadinii***	***P. lynferdii***	***Rhodosporidium toruloides***	***Rhodotorula pilati***	***Sporidiobolus ruineniae***	***Zygosaccharomyces bailii***
1	D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	D-Galactose	+	+	+	+	+	+	+	+	+	+	+	+	-(+)	-	-(+)	+	+	+	+	+
3	L-Sorbose	+	-	-	+	+(-)	+	v	+(-)	+	+(-)	v	+	+	w/-	-	+(-)	+	-	+	+
4	D-Glucosamine	n	+	-	-	+	v	n	n	+	n	n	v	-	n	n	n	+	-	n	n
5	D-Xylose	v	+(-)	+	+	v	v	v	+(-)	+	-(+)	v	v	+	+	+	v	-	-	+	v
6	L-Arabinose	+	n	+	n	v	+	-	v	n	+(-)	+	+	+	+	+(-)	-(+)	+	+	n	n
7	D-Arabinose	-	n	+	n	v	+(-)	-	-(+)	n	+(-)	v	-	v	w/-	-(+)	-(+)	+	+	n	n
8	L-Rhamnose	-	+	w/-	+(-)	-	v	v	v	+	+	v	v	+(-)	+	-	-	+(-)	-	n	+
9	Sucrose	+	n	+	n	+	+	+	+	+	+	+	-	+	+	+	+(-)	+	+	+	-
10	Maltose	+	+	+	v	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
11	α,α-Trehalose	+	n	n	+	+	n	n	+	n	n	n	n	+	n	+	+	n	+	n	n
12	Methyl α-D-glucoside	+	+	-	n	+	n	n	+	n	n	n	n	+	+	v	+(-)	n	n	n	+
13	Cellobiose	+		+	+	+	n	+	+	+	n	n	n	+	w/+	+	+	n	+	n	-
14	Arbutin	n	n	n	n	+	n	n	+	n	n	n	n	v	n	+	+	n	n	+	n
15	Melibiose	-	+	+	n	+	v	+	v	-	-(+)	-(+)	-(+)	v	n	-	-	+	-	+(-)	-

Table 3. (Cont'd.)

16	Lactose	v	+	+	n	-(+)	v	+	+(-)	+	-	-	-	-(+)	-	-	-(+)	v	-	+(-)	n
17	Raffinose	-	+	+	-	+	+	+	+	v	+	+	+	+	w/-	+	+	v	-	+	+
18	Melezitose	+	+	+(-)	+	+	+	n	+	+	+	+	+	+	+	+	+(-)	+	+	-(+)	n
19	Inulin	n	-	-	n	v	n	n	v	n	n	n	n	-	n	v	+	n	n	n	n
20	Starch	+	n	+(-)	n	-	v	n	-	-	+	+	+	+	-	-	-(+)	+	+	n	n
21	Glycerol	n	n	n	n	n	n	+	+	+	n	n	n	n	n	n	n	n	n	+	-
22	Erythritol	-	-	-	+	-	v	-	-(+)	-	-	v	-	-	-	-	+	-	-	-	-
23	Xylitol	n	n	n	n	+(-)	n	+(-)	+(-)	-(+)	n	n	n	-	+	+(-)	v	n	n	n	-
24	L-Arabinitol	n	+	n	n	n	n	v	v	+	n	n	n	n	n	n	n	n	n	+	-
25	D-Glucitol	+	n	n	n	+	+	n	n	n	n	n	n	n	+	n	n	n	n	n	n
26	D-Mannitol	n	n	+	+	+(-)	+	+	v	+	+	+	+	+	n	v	v	+	+	n	+
27	Galactitol	+	+	+(-)	+	+	v	n	n	+	+(-)	v	v	-(+)	n	n	n	-(+)	-	+	n
28	*Myo*-inositol	n	+	+	-	+	+	n	-	v	n	+	+	n	-	n	-	n	-	-	n
29	2-Keto-D-gluconate	-	n	n	+	n	n	n	+	n	n	n	n	+	-	n	n	-	n	-	-
30	5-Keto-D-Gluconate	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	-	n	n	n
31	D-Gluconate	n	n	n	+	v	+	+	v	-	n	n	n	-	+	-	-(+)	n	n	n	n
32	D-Glucuronate	-	v	+	n	n	+	n	n	n	+(-)	+	+	-	n	n	n	-(+)	+	-	n
33	D-Galacturonate	n	+	n	+	-(+)	n	n	-	-	+	+	n	-(+)	n	-	-(+)	-	n	n	n
34	DL-Lactate	n	+	-	+	+	n	+(-)	+	n	n	n	n	+	+	+	+	n	n	n	-
35	Succinate	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+
36	Citrate	+	+	+	+	+	-(+)	+	+	+	-	v	-	-(+)	+	+	+	-	-	+	-
37	Methanol	n	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-(+)	-	-	-	-
38	Ethanol	+	+(-)	+	+	+	+(-)	v	n	+	+	+	-	+	n	+	+	+(-)	+	+	+
39	Propane 1,2-diol	n	n	n	n	n	n	v	-(+)	v	n	n	n	n	n	n	n	n	n	n	n
40	Butane 2,3 diol	n	n	n	n	n	n	n	-	n	n	n	n	n	n	+	+	n	n	n	n

Responses: + = positive; +(-) = mostly positive with some negative; -(+) = mostly negative with some positive; w/- =weak or negtive, w/+ =weak or positive, - = negtive, n=not determined

Table 4. Physiological/biochemical tests of yeast species other than assimilation of carbon campounds.

		Yeast species																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
No.	**Physiological/Biochemical tests**	***Bensingtonia phyllada***	***Bullera pyricola***	***B. pseudoalba***	***Candida succiphila***	***C. valdiviana***	***Cryptococcus albidus***	***Debaryomyces castellii***	***D. hansenii***	***D. yamadae***	***Fibulobasidium inconspicuum***	***Filobasidiella neoformans***	***Filobasidium uniguttulatum***	***Phaffia rhodozyma***	***Pichia euphorbiae***	***P. jadinii***	***P. lynferdii***	***Rhodosporidium toruloides***	***Rhodotorula pilati***	***Sporidiobolus ruineniae***	***Zygosaccharomyces bailii***
	Fermentation																				
1	D-Glucose	n	n	n	+	n	n	+	-	v	n	n	v	n	+	+	+	-	n	n	+
2	D-Galactose	n	n	n	+(-)	n	n	n	+	+	n	n	n	n	n	n	n	n	n	n	-
3	Methyl α-D-glucoside	n	n	n	n	-(+)	n	v	-(+)	n	n	n	n	-	n	w/-	v	n	n	n	n
4	Sucrose	n	n	n	n	-(+)	n	+	v	-	n	n	n	-	n	v	v	n	n	n	n
5	Inulin	n	n	n	n	w/-	n	+(-)	-(+)	-	n	n	n	w/-	n	w/-	v	n	n	n	+
	Assiml. of N comp.																				
1	Nitrate	+	+	-	w/-	+	v	n	-	n	-	-	-	-	-	+	+	+(-)	+	+	-
2	Ethylamine	-	-	n	+	n	-(+)	n	n	n	v	v	+	+	n	+	+	+(-)	+	n	n
3	L-Lysine	n	n	n	-	+	+	n	+	n	n	n	n	+	n	+	+	n	n	n	+
4	Cadaverine	+	+	+	n	+(-)	-(+)	+	+	v	+	v	v	+	+	+	+(-)	+	+	+	+
5	Glucosamine	-	-	+	n	+(-)	-(+)	v	v	+	v	v	v	-(+)	n	+	-(+)	v	+	n	-

Table 4. (Cont'd.)

	Growth without vitamin(s)																			
1	Without vitamins	n	n	n	v	n	-	+	+(-)	-(+)	n	n	n	+	n	+	n	+	-	+	+
2	Without *myo*-Inositol	n	n	n	n	+	n	+	+	+	n	n	n	+	n	+	+	n	n	n	+
3	Without Biotin	n	-	n	n	+	n	n	+	n	n	n	n	+	n	+	+	n	n	+	n
4	Without Thiamin	-	+	n	+	+	-(+)	n	+	+	+	+	+	+	+	+	+	+	+	+	n
5	Without Biotin & Thiamin	n	n	+	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	+	n
6	Without Pyridoxine	n	n	n	n	+	n	n	+	n	n	n	n	+	+	+	+	+	n	n	n
7	Without Thiamin & Pyridoxine	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
8	Without Niacin	n	n	+	n	+	n	+	+	+	n	n	n	+	n	+	+	n	n	n	+
9	Without *p*-aminobenzoate	n	+	+	n	n	+	n	n	n	+	+	+	+	n	n	n	+	+	n	n
	Growth at diff. temp.																				
1	At 25°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	At 30°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	At 35°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	At 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	At 40°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Additional tests																				
1	With 0.01% (w/v) cycloheximide	n	n	n	v	n	n	n	-	n	n	v	n	n	n	n	n	n	n	n	n
2	With 0.10% (w/v) cycloheximide	n	n	n	n	+	n	n	-	+	n	n	n	n	n	n	n	.-	n	n	-
3	With 1% Acetic Acid growth	n	n	n	-	-	n	v	-(+)	v	n	n	n	-	n	-	-	n	n	n	+
4	With 50% (w/v) D-glucose	n	n	n	+	+	n	n	v	+	n	n	+	+	+	+(-)	+	n	n	n	+
5	With 60% (w/v) D-glucose	n	n	n	-	-	n	v	v	+	n	n	n	-	n	v	+(-)	n	n	n	-
6	Starch formation	n	-	-	n	n	+(-)	n	n	-	-	-	-	+(-)	n	n	n	-	-	n	n
7	Diazonium Blue B reaction	n	-	n	n	n	-	n	-	-	v	+	n	+	n	n	n	+	n	n	n

Responses: + = positive; +(-) = mostly positive with some negative; -(+) = mostly negative with some positive; w/- =weak or negative, w/+ =weak or positive, - = negative, n=not determined

Cultivated soil yielded lesser number of yeast species as compared to garden soil. Univariate ANOVA of yeast species from soil revealed that their occurrence was significantly different ($p<0.001$) in cultivated and garden soil. Bonferroni test also confirmed significant differences among yeast species. The population of yeast ranged form $9.3x10^4$ to $0.23x10^4$ colony forming unit (cfu)/g soil. The population of yeast cells in soil is greatly dependent upon the type of nutrients reaching them. Garden soil showed greater occurrence of yeast probably due to presence of different nutrients from dead and decaying plant parts as compared to cultivated field. Competition for nutrients is probably the single most important factor in yeast ecology. Among physiochemical factors that affect the ecology of yeasts, most important appear to be the energy sources, nutrients, temperature, pH value and water (Rose & Harrison, 1987-1993).

In the present study we also isolated some of the economically important yeasts e.g., *Pichia jadinii* in its imperfect state (*Candida utilis*), used for the production of feed yeast in animal diets. It does not require added vitamins and has the ability to utilize ammonium or nitrate nitrogen and many carbon sources including Cellobiose and D-xylose and grows at 37°C. Similarly methylotrophic yeast, *Candida succiphila*, is used for the production of single cell proteins (SCP). Contrary to strains used in industry, we isolated strains of *Phaffia rhodozyma* that is able to grow at high temperatures of up to 40°C. This pigmented and fermentative yeast has potential in biotechnology because of its carotenoids contents mainly astaxanthin that costs about $ 2400/Kg (Andrewes *et al*., 1976). This pigment is responsible for the orange to pink color of salmonid flesh and the reddish color of boiled crustacean shells. Lack of information on yeast flora from Pakistan soil would suggest that there is need to evaluate yeast flora from different habitats that could include industrially useful species as well.

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(Received for publication 15 March 2003)